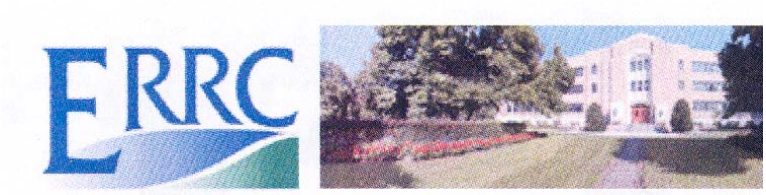

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Title: Application of the Vacuum/Steam/Vacuum Surface Intervention Process to Reduce Bacteria on the Surface of Fruits and Vegetables

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Application of the vacuum/steam/vacuum surface intervention process to reduce bacteria on the surface of fruits and vegetables ☆

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Abstract

A vacuum/steam/vacuum (VSV) surface intervention process has previously been developed for poultry and hot dogs. The process uses a brief exposure to vacuum to remove surface air and water to expose bacteria. After a short treatment with saturated steam (0.1 s), a second vacuum treatment evaporatively cools the surface, resulting in the destruction of bacteria with little or no thermal damage. The VSV surface intervention process has also been applied to fruits and vegetables. Optimization methods were used with cantaloupes, grapefruits, and beets to determine process conditions for steam temperature, steam time, vacuum time, and number of cycles to destroy bacteria with the constraint of little or no thermal damage. Inoculated *Listeria innocua* was used for the cantaloupe and grapefruit studies and total aerobic plate count (APC) was used for the beet study. Bacteria destruction ranged from 2.5 log cfu/ml APC for beets to almost 4 log *L. innocua* for grapefruits. The process was successfully applied to other fruits and vegetables such as papayas, mangoes, avocados, kiwis, carrots, cucumbers, and peaches, using the nominal process conditions found with cantaloupes, grapefruits, and beets. Applying the process to bananas, cauliflower, broccoli, and peppers resulted in thermal or mechanical damage. The total process time was 0.5–1.2 s, depending on the number of cycles and the process time per cycle. Assuming that these results with APC and *L. innocua* are indicative of the treatment of naturally present pathogens, this surface intervention process should ensure that fruits and vegetables suitable for this process will reach the consumer having greatly reduced levels of bacterial contamination.

Keywords: Cantaloupe; Grapefruits; Papaya; Beets; Intervention process; Tropical fruits; *Listeria*

Industrial relevance: Food safety improvement is of continuous industrial concern. The process presented is an ingenous surface intervention process aimed at destroying bacteria on food surfaces. The process consists of vacuum/steam/vacuum (VSV) treatment to remove mass transfer barriers (air, water) from the products surface to allow a more effective steam treatment. Additionally the final vacuum process provides a surface cooling effect and thus reducing thermal damage of the products. The VSV intervention process was shown to effectively (3 to 5 log cfu/ml) destroy bacteria, *L. innocua* and total aerobic plate count but successful application of the process was product specific.

☆ Mention of brand or firm names does not constitute an endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

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1. Introduction

According to the Center for Disease Control (CDC) Emerging Infections Program, the Foodborne Diseases Active Surveillance Network (Anon., 1999), millions of people get food poisoning every year but only a small fraction of these cases are ever confirmed because most never seek medical help or submit laboratory samples for identification. During 1998, the most commonly detected organisms were *Campylobacter* with 4031 cases and *Salmonella* with 2849 cases (Anon., 1999). There were 106 confirmed cases of Listeriosis. The incidence rate for campylobacteriosis was 21.7 per 100 000 population, and for salmonellosis, was 12.4 per 100 000 (Anon., 1999). Marsili (1998) estimated that there are 4400–6300 deaths annually in the US caused by pathogens. Approximately 1000–2000 deaths occur due to *Salmonella*, 270–510 from *Listeria monocytogenes*, 200–730 from *Campylobacter*, and 50–100 from *E. coli* O157:H7.

We have been developing a process called the Vacuum/Steam/Vacuum (VSV) Surface Intervention process for destroying bacteria on the surface of food, especially raw food. A prototype surface intervention processor was designed, fabricated, and patented (Morgan, Goldberg, Radewonuk & Scullen, 1996; Morgan, Radewonuk & Scullen, 1996; Morgan, 1994). Optimal processing conditions for destroying bacteria on the surface of chicken were developed (Kozempel, Goldberg, Radewonuk & Scullen, 2000a). The surface intervention process was also applied to hot dogs (Kozempel, Goldberg, Radewonuk & Scullen, 2000b) and optimum process conditions were determined. Cycling the treatment significantly improved the microbiological destruction.

Morgan et al. (1996) presented an excellent discussion of the theory behind the VSV surface intervention process. In brief, steam is capable of destroying pathogens. However, air and water on the surface of the product act as insulation (Morgan & Carlson, 1960; Perry, Chilton, & Maloney, 1984). Although water is a good conductor of thermal energy, relative to steam, it acts as insulation. Exposing the product to steam for a sufficient amount of time to transfer the energy across the air/water barrier to destroy bacteria thermally damages the surface. VSV surface intervention employs a short exposure to vacuum to remove these insulating fluids. This is followed by a quick burst of condensing steam that rapidly transfers the energy directly to the bacteria. Then a second exposure to vacuum evaporatively cools the product surface, preventing thermal damage. Although the interfering surface layers are removed with the initial application of vacuum, the condensing steam itself continuously deposits an insulating water (condensate) layer during processing. Cycling between vacuum and steam effectively removes this redeposited water layer as soon as it forms and improves surface treatment. The process time is of the order of 1–2 s. For example, the process conditions for whole chicken carcasses were steam time of 0.05 s at 138 °C for three cycles or steam for 0.1 s for two cycles. Destruction of 1–2 logs of inoculated *Listeria innocua* were achieved (Kozempel et al., 2000a). Cycling with steam at 138 °C for 0.3 s and vacuum for 0.1 s achieved a 5-log destruction of inoculated *Listeria innocua*, which is frequently considered to be pasteurization (Kozempel et al., 2000b).

The CDC reported that, between 1973 and 1987, among food borne outbreaks, 2% of the outbreaks and 2% of the outbreak-associated cases were connected with fresh produce. Between 1988 and 1991, these numbers increased to 5 and 8%, respectively (Tarr, Besser, Hancock, Keene & Goldoft, 1997). In 1989, 25 000 were infected by *Salmonella* from cantaloupes. Over 1000 were sickened with *Cyclospora* from raspberries in both 1996 and 1997. de Simon, Tarrago & Ferrer (1992) found that 7.8% of 103 samples of vegetables tested carried some species of *Listeria*.

The bacterial contamination of raw fruits and vegetables should be predominantly on and in the surface. Fruits and vegetables are usually washed to remove dirt and the accompanying bacteria. However, simple water washing has minimal effect in removing the bacteria in the surface of produce. Various antimicrobial washes have been tried with some measure of success. The drawbacks are the cost and stigma associated with using chemical washes. Cherry (1999) lists many of these chemical disinfectants. For example, chlorine destroys 1–2 log, hydrogen peroxide 3 log, ozone 1–3 log, and trisodium phosphate 4 log. Steam, as well as hot water, has been used. Tottenham and Purser (2000) report the use of steam treatment at 74 °C for up to 2 min which results in a 5-log reduction in bacteria. However, the fruit or vegetables are then not suitable for fresh market sales.

Since the VSV surface intervention process is designed to destroy bacteria in the surface of raw food, we hypothesize that the process will destroy surface bacteria on raw fruits and vegetables. The VSV process offers the advantages of using only water, is fast (1–2 s), and causes little or no thermal damage. The primary objective of this work is the adaptation of the VSV surface intervention process for inactivating bacteria on raw fruits and vegetables, thus enhancing the microbial safety of raw produce and expanding the usefulness of the VSV process. The specific objective is the application of the VSV process to fruits and vegetables using naturally present aerobic plate count (APC), when sufficiently high, or inoculated *L. innocua* as non-pathogenic surrogate bacteria.

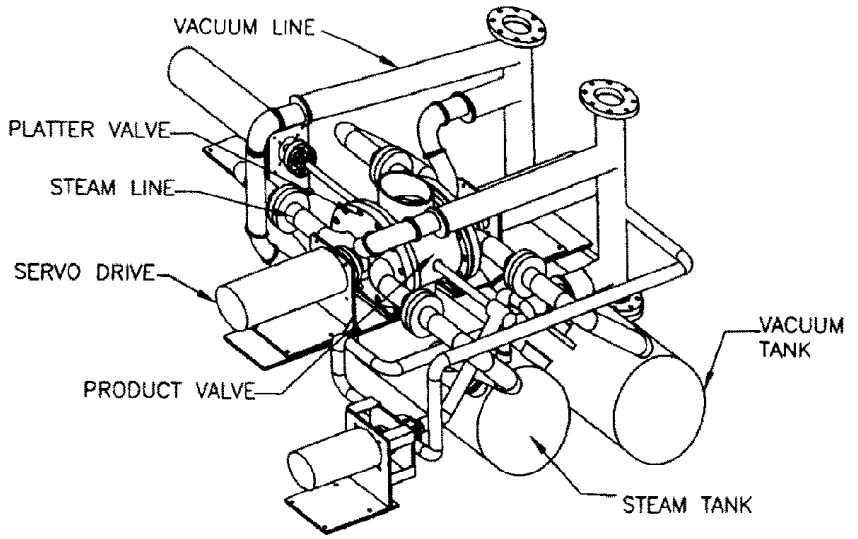

Fig. 1. Schematic diagram of the prototype VSV surface intervention processor.

2. Materials and methods

2.1. VSV surface intervention processor mechanical design

The surface intervention processor was designed to process chicken carcasses, specifically broilers. The performance requirements of a surface intervention processor for chicken are to accept carcasses individually and to enclose them in a chamber within a rotor; to evacuate that chamber; to treat it in that closed chamber with steam; to cool it with vacuum; and finally to eject it into a clean environment. The simplest design, one chamber in one rotor, was designed and constructed. Fig. 1 shows a schematic diagram of the processor and Fig. 2 shows details of the product treatment section. A cylindrical chamber for a broiler carcass should be approximately 200 mm in diameter, and 240 mm deep. Such a chamber is provided by an 8-inch ball valve. This same cylindrical chamber (product valve) was used to treat individual fruits and vegetables. Obviously, a different size and shape product valve would be used in a machine designed specifically to treat fruits and vegetables instead of chicken carcasses.

To admit vacuum or steam into the closed chamber, two opposed 200-mm holes were bored through the stator at right angles to both the axis of rotation of the ball and to the centerline of the open chamber. Two gas valves were close coupled to these 200 mm ports and consisted of a flat disk rotating against an inlet header, which held PEEK (polyetheretherketone) seals. Each disk contained two holes, which, when stopped at one of the ports in the inlet header, permitted gas to flow into the treatment chamber. Multiple holes reduced the rotor angular movement necessary for valve action and increased the cross-sectional area for gas flow. Each disk was programmed independently and moved by its own servo-motor. The servos were 50 J units, capable of high acceleration and deceleration.

In order to expose all exterior surfaces of the test specimen to treatment, a screen was installed at the mid-point of the product valve to hold the sample. The steam generator was charged with deionized water and

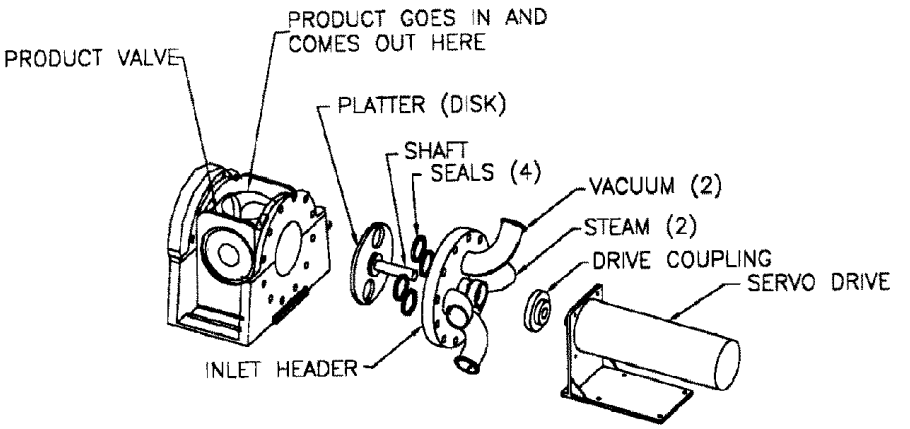

Fig. 2. Details of the product treatment section of the prototype VSV surface intervention processor.

boiled for 30 min for de-aeration. The vacuum receiver was adjusted to 100 mbar and its condenser coil cooled to 4 °C.

2.2. VSV surface intervention processor operation

Each sample (fruit or vegetable) was manually inserted into the treatment chamber of the surface intervention processor. A servo was used to rotate the ball valve 90° to seal the chamber from the outside atmosphere. The operation of the ball valve was computer controlled. The platter valves were rotated to expose the sample to vacuum, then steam, and then vacuum again. With multiple cycles, the sequence of vacuum then steam was repeated multiple times. The process variables studied were steam temperature, steam time; initial, intermediate, and final vacuum times, and number of cycles. The vacuum was 100 mbar. After treatment, the ball valve was rotated back 90° to expose the sample to atmosphere. The fruit or vegetable sample was aseptically removed by hand with a latex exam glove after treatment.

2.3. Fruit and vegetable preparation

Fresh produce was purchased from local supermarkets and stored in a refrigerator until use, normally up to 2 days. Every effort was made to secure produce without surface treatments. However, all grapefruits purchased were coated with a vegetable wax. Papayas were coated with a food grade vegetable wax and thiobendazole, a fungicide. We entertained the idea of removing the coating by a solvent rinse. However, this would not represent a valid representation of actual commercially available produce. We decided that the best course of action was to use the fruits as they came from the market. This should more closely simulate post-harvest contamination. To test pre-harvest contamination, it would be necessary to acquire these products before the coating was applied.

Because we were hoping for bacteria reductions up to 5 log with the VSV process, the produce were inoculated with *L. innocua* to have controls with bacteria counts greater than 5 log cfu/ml. *L. innocua* was chosen because it is non-pathogenic and has similar or higher resistance than *L. monocytogenes* (Ryser & Marth, 1999). The exceptions were beets and carrots, which were not inoculated because we felt the bacterial levels were already sufficiently high to determine bacteria destruction: 5.71 log cfu/ml APC for carrot control and 5.83–6.00 log cfu/ml APC for beets. For these vegetables, a total APC was used. For the rest of the produce that was inoculated, the native bacterial counts were very low (< 2.5 log cfu/ml), especially relative to the inoculated bacteria, *L. innocua*. The exception was cucumbers, which had a native bacteria count of 4.55 log cfu/ml APC. Cucumbers were inoculated to get control samples of 5.4 log cfu/ml. However, all bacteria were counted as APC and *L. innocua*.

To prepare an inoculum of *L. innocua*, a loopful of culture was removed from a refrigerated slant and added to a 100-ml brain–heart infusion broth (BHI; Difco Laboratories, Detroit, MI) supplemented with 3% glucose. Inoculated BHI was incubated overnight at 28 °C. The amount of inoculum, contact and drain times were chosen by trial and error to raise the bacteria count on the sample to a sufficient level for experiments, > 5 log. If the counts on the inoculated control samples were lower than desired, the time for inoculation was increased in subsequent experiments. The samples were inoculated by dipping into a container with 10^6 cfu/ml *L. innocua* for 5–60 min. Specifically, mangoes, avocados, kiwis, peaches, and cucumbers were dipped for inoculation for 10 min. Papayas were dipped for 60 min. Cantaloupes were initially dipped for 5 min, but in subsequent experiments, they were dipped for 10 min. Grapefruits were progressively dipped for longer times, 10, 30 and 60 min, in subsequent experiments. Upon removal, the fruits and vegetables were allowed to drain and dry in ambient air for 60 min before experimentation. This procedure was previously found (unpublished data) to give high surface counts when inoculating chicken.

2.4. Microbiology testing

To determine *L. innocua* counts after processing in the intervention processor, the sample was placed in a sterile plastic bag with Butterfield buffer solution (Difco Laboratories) and manually rinsed for 60 s (60 shakes). The volume of buffer used was adjusted for each product to approximate a constant ratio of buffer volume to sample volume. Therefore, for grapefruits and cantaloupes, 400 ml of buffer were used per sample. Two hundred ml were used for beets, carrots and cucumbers and 150 ml for mangoes. For papayas, avocados, and peaches, 100 ml were used. Fifty ml were used for kiwis.

The rinses were appropriately diluted with sterile 1% peptone water and plated onto Tryptose Agar (Difco Laboratories) using a spiral plater. Tryptose Agar is a general agar and would promote the growth of most bacteria. We used Tryptose Agar in order to pick up all bacteria, injured, non-injured, and possibly background. It is not specific for *Listeria*. However, the native bacteria counts were always very low relative to inoculated bacteria.

The plates were incubated at 37 °C for 1 day. Colonies were counted and expressed as cfu/ml (colony forming units/ml). Additional aliquots of serial dilutions were plated on Petrifilm™ Aerobic count plates (3 M Microbiology Products, Minneapolis, MN, USA) for aerobic plate counts (APC) following the manufacturer's

Table 1
2^3 Experimental design and ANOVA for cantaloupe

Source	−	+
A	138	143
B	0.10	0.25
C	0.10	0.25

1 cycle

Source	Mean Log cfu/ml	*L. innocua*	
		Mean square	*F* value
A	1.90	3.361	5.57*
B	2.51	1.819	3.02
AB	2.19	0.498	0.82
C	2.92	0.210	0.35
AC	2.41	0.197	0.33
BC	2.14	0.636	1.05
ABC	1.67	0.444	0.74
Error		0.604	

A = steam temperature, °C; B = steam time, s; C = Initial vacuum time, s; Final vacuum time = 0.3 s. Treatment samples consisted of four replicates. Significant differences represented by; $^*P \leq 0.05$. Control (six replicates) = 5.44 log cfu/ml. Inoculate 10 min, ambient dry 60 min.

recommended procedures. The Petrifilms™ were manually counted after incubation at 37 °C for 48 h. All dilutions were plated in triplicate.

2.5. Process optimization

For optimization studies, 2^2 and 2^3 factorial experimental designs (Davies, Box, Cousins, Hilmsworth & Sillitto, 1960) were used. Treatment samples consisted of four replicates, except for three replicates for Tables 1 and 2. The data from the factorial designs were analyzed by analysis of variance using the replicate within treatment terms as error terms. Control samples of inoculated fruits and vegetables were taken to provide an independent estimate of the extent of bacteria destruction. A null hypothesis (Volk, 1958) was made on the difference between means (H_o; $\text{mean}_1 = \text{mean}_2$) to compare the mean bacteria counts at various process conditions.

Table 2
2^2 Experimental design and ANOVA for cantaloupe

	−	+
A	0.1	0.25
B	1	2

Source	Mean Log cfu/ml	*L. innocua*	
		Mean square	*F* value
A	2.78	0.278	0.41
B	1.86	5.676	8.29*
AB	1.43	0.107	0.16
Error		0.6844	

A = steam time, s; B = # of cycles; Initial vacuum time = 0.1 s; Final vacuum time = 0.3 s; Steam temperature = 143 °C. Treatment samples consisted of four replicates. Significant differences represented by; * $P \leq 0.05$. Control (six replicates) = 5.60 log cfu/ml. Inoculate 10 min, ambient dry 60 min.

3. Results and discussion

Details of the research will be presented for three different types of agricultural crops that are subject to different vectors of bacterial contamination. The first is cantaloupe, which grows on the ground. Next is grapefruit, which grows in trees and should not have direct contact with the ground. Beet is the third crop, and it grows in the ground. Following this, results of applying the process to other fruits and vegetables will be presented. Optimization procedures were used with the constraints that there be essentially no thermal damage to the product and that the process time be less than 2 s.

3.1. Cantaloupes

We chose cantaloupes and a broad range of process parameters for the first experimental design to be sure to encompass an optimum region approximating the constraint boundaries. Factorial designs were used. Initial experiments were made using one cycle. After choosing an optimum within the constraints of the study for one cycle, cycling was added to the study.

In the first 2^3 factorial design (data not shown), the design factors, steam temperature, steam time, and vacuum times were used. The cantaloupes were inoculated with *L. innocua* by immersion for 5 min followed by ambient air drying for 1 h. The steam times were 1.0 s for the high or + value and 0.25 s for the low or − level. The vacuum times were 2.0 s for the high or + value and 0.50 s for the low or − level. They exceeded our time constraint for the process, but were later shortened to satisfy the constraint. Three replicates were used.

As expected, steam temperature was statistically highly significant ($P \leq 0.001$), indicating the higher temperature of 143 °C is nearer the optimum temperature. Steam time was statistically significant ($P \leq 0.05$) indicating the optimum is greater than 1.0 s, which is outside the time constraint. The vacuum times were not statistically significant. The control using three replicates was 5.39 log cfu/ml *L. innocua*. For the high level of steam temperature (143 °C), the mean count for *L. innocua* was 0.67 log cfu/ml ($n = 12$) and for the high level of steam time (1.00 s) the mean count for *L. innocua* was 1.43 log cfu/ml ($n = 12$). The reduction of *L. innocua* was 4.7 and 4.0 log, respectively.

Design 1 was exploratory to determine the range to study. The next design (data not shown) fine-tuned the

search somewhat. The cantaloupes were inoculated with *L. innocua* by immersion for 5 min followed by ambient air drying for 1 h. Steam temperature was set to 127 or 138 °C and the steam times were shortened to 0.25 or 0.75 s because of the time constraint. Since vacuum times were not significant in the previous design, they were shortened to 0.25 and 0.5 s.

Steam temperature was statistically highly significant; 138 °C was better than 127 °C. There was no significance to the steam time indicating that the shorter time period can be used. In this design, the shorter vacuum time was statistically significantly better. The mean log count at the high or + level of steam temperature was 2.06 log cfu/ml *L. innocua* ($n = 12$). The mean log count at the low or − level of vacuum time was 2.14 log cfu/ml *L. innocua* ($n = 12$). The control using three replicates was 5.60 log cfu/ml *L. innocua*. Therefore, the reduction in *L. innocua* was 3.54 log at 138 °C (A +) and 3.46 log at 0.25 s vacuum (C −).

Steam temperature was significant in both previous designs, so the high or + value was increased in the third design, Table 1, to 143 °C. This is a practical limit. Above this temperature, this prototype VSV processor experienced leaks and intermittent operating problems. Both steam and initial vacuum times were reduced to 0.1 and 0.25 s for the low (−) and high (+) conditions. Our previous experience with other products has shown that it is best to keep the final vacuum time at or above 0.3 s. Thermal damage can occur if the time for evaporative cooling is too short. Final vacuum time was set to 0.3 s. The cantaloupes were inoculated with *L. innocua* by immersion for 10 min followed by ambient air drying for 1 h.

Table 1 lists the results of the third factorial design. The top of the table lists the 2 levels of the design factors, steam temperature, steam time, and initial vacuum time. In the lower part of the table, the first column, Source, identifies the variables and variable combinations that were at the high or + level. Column 2 presents the experimental results. Column 2 presents the mean of the replicates at each set of experimental conditions. Four replicates were used in Table 1. The third column presents the mean square for each factor or combination of factors. The error (last row of column 3) is the residual mean square for calculating the F value in column 4 for the individual factors and combination of factors.

As shown in Table 1, the only statistically significant variable was steam temperature. The mean count at the high (A +) level of steam temperature (143 °C) was 1.90 log cfu/ml *L. innocua* ($n = 16$). The control using six replicates was 5.44 log cfu/ml *L. innocua*. Therefore, the reduction in *L. innocua* was 3.54 log cfu/ml at 143 °C (A +). Therefore, the optimum process parameters chosen for one cycle within the time constraint were 143 °C steam for 0.1 s and an initial vacuum time of 0.1 s. Steam temperature higher than 143 °C may be closer to the optimum but this is the practical upper limit for this prototype VSV processor.

In Table 2, cycling was added in a 2^2 design. The top of the table lists the two levels of the design factors, steam time and number of cycles. Steam temperature was 143 °C. In the lower part of the table, the first column, Source, identifies the variables and variable combinations that were at the high or + level. Column 2 presents the experimental results. Column 2 presents the mean of the replicates at each set of experimental conditions. Four replicates were used. The third column presents the mean square for each factor or combination of factors. The error (last row of column 3) is the residual mean square for calculating the F value in column 4 for the individual factors and combination of factors.

As expected, two cycles were statistically significantly better than one cycle. Steam time was not statistically significant. The mean count at the high or + level of number of cycles (two cycles) was 1.55 log cfu/ml *L. innocua* ($n = 8$). The control using six replicates was 5.60 log cfu/ml *L. innocua*. Therefore, the reduction in *L. innocua* was 4.05 log cfu/ml at (B +). Three cycles were also compared, but there was no statistically significant difference between two and three cycles.

There was a concern that if the cantaloupe-inoculated bacteria were not processed immediately, the bacteria may form biofilms or become more strongly attached to the surface and more difficult to destroy. To determine if this is a problem, another 2^2 factorial design was made in which half of the cantaloupes were processed immediately after inoculation and ambient air drying, and the other half after 2 days cold storage in a refrigerator. Table 3 lists the results. The variable levels and results are listed as described for Tables 1 and 2. Steam temperature was 143 °C. Five replicates were used. There was no statistically significant difference due to storage. The count after treatment after 2 days was 2.20 vs. 2.09 log cfu/ml *L. innocua* for treatment immediately after inoculation and drying. There was a statistically significant ($P \leq 0.05$) difference for two cycles (1.90 log cfu/ml *L. innocua*) vs. one cycle (2.39 log cfu/ml *L. innocua*). No other single independent variable was statistically significant.

The optimum process parameters for cantaloupes within the time constraint were chosen as steam temperature of 143 °C for 0.1 s, initial vacuum time of 0.1 s and two cycles. Final vacuum time was 0.3 s. At these conditions, there was no visible thermal damage to the fruit.

3.2. Grapefruits

As previously mentioned, grapefruits came from the

Table 3
2^2 Experimental design and ANOVA for cantaloupe, effect of storage

	−	+
A	1	2
B	0	2

Source	Mean Log cfu/ml	*L. innocua*	
		Mean square	*F* value
A	2.09	1.205	6.80*
B	2.69	0.0616	0.35
AB	1.70	1.245	7.03*
Error		0.1772	

A = # of cycles; B = # of days storage at 4 °C; Initial vacuum time = 0.1 s; Final vacuum time = 0.3 s; Steam temperature = 143 °C. Treatment samples consisted of five replicates. Significant differences represented by; $*P \leq 0.05$. Control (10 replicates) = 5.37 log cfu/ml. Inoculate 10 min, ambient dry 60 min.

supermarket coated with vegetable wax. Therefore, the application of the VSV process on these products represents intervention of fruit infected after post-harvest treatment. To study fruit before post-harvest processing and the addition of the wax coating will require locating a source of uncoated grapefruits.

In the first 2^3 factorial design for grapefruits (data not shown), the design factors, steam temperature, steam time and final vacuum time were used. Based on the results for cantaloupes and to keep the times within the time constraint of 2 s, the values for the process parameters for grapefruits for the first experimental design were set over a more narrow range. The steam temperatures were 138 °C for the high or + value and 127 °C for the low or − level. The steam times were 0.20 s for the high or + value and 0.10 s for the low or − level. The final vacuum times were 0.50 s for the high or + value and 0.25 s for the low or − level. The grapefruits were inoculated with *L. innocua* by immersion for 10 min followed by ambient air drying for 1 h. Four replicates were used. Initial vacuum time was 0.1 s.

There was a highly statistically significant ($P \leq 0.01$) difference between 127 and 138 °C steam temperature. The higher temperature was better. There was no statistically significant difference between 0.1 and 0.2 steam times or between 0.25 and 0.50 s final vacuum times. The mean log count at the high or + level of steam temperature was 0.50 log cfu/ml *L. innocua* ($n = 16$). The control using five replicates was 2.25 log cfu/ml *L. innocua*. Therefore, the reduction in *L. innocua* was 1.75 log at 138 °C (A +).

In the next design, Table 4, the temperature was increased to 143 °C and the steam time reduced to 0.05 s. Final vacuum time was also reduced to 0.1 s to see if there was a statistically significant effect, even though there might be thermal damage. There was no visual thermal damage. The variable levels and results are listed as in Table 1.

Both steam temperature and steam time were statistically significant, steam temperature at $P \leq 0.001$ and steam time at $P \leq 0.05$. These results indicate that the optimum process temperature is 143 °C or higher and the steam time is 0.1 s. Although there was no statistically significant difference in the final vacuum times, 0.3 s was chosen to be sure of no thermal damage. Initial vacuum time was 0.1 s. The mean bacteria count at the high level of steam temperature (A + , 143 °C) was 0.43 log cfu/ml *L. innocua*. The mean bacteria count at the high level of steam time (B + , 0.25 s) was 0.88 log cfu/ml *L. innocua*. The mean bacteria count of the control (four replicates) was 4.14 log cfu/ml *L. innocua*. The mean bacteria destruction at the high level of steam temperature was 3.71 log cfu/ml *L. innocua*, and at the high level of steam time was 3.26 log cfu/ml *L. innocua*.

Cycling the vacuum and steam should increase bacteria destruction. Cycling was checked at both the optimum conditions chosen (143 °C, steam time = 0.1 s, initial and intermediate vacuum times = 0.1 s, and final vacuum time = 0.3 s) and at the lower temperature of 138 °C. One, two and three cycles were tested. Four replicates were used. The grapefruits were inoculated with *L. innocua* by immersion for 60 min followed by ambient air drying for 1 h. The count for the control samples was 4.20 log cfu/ml *L. innocua* (four replicates). The bacteria count of treated samples was below the detection limit, 0.6 log cfu/ml *L. innocua*, for all cases except for one cycle at 138 °C for which the count

Table 4
2^3 Experimental design and ANOVA for grapefruits

	−	+
A	138	143
B	0.05	0.10
C	0.10	0.25

Source	Mean Log cfu/ml	*L. innocua*	
		Mean square	*F* value
A	1.47	18.88	43.01***
B	2.07	3.328	7.58*
AB	< 1.32	0.106	0.24
C	2.57	0.572	1.30
AC	1.57	0.001	0.003
BC	1.44	1.133	2.58
ABC	< 1.32	0.852	1.94
Error		0.439	

A = steam temperature, °C; B = steam time, s; C = final vacuum time, s; Initial vacuum time = 0.1 s. Treatment samples consisted of four replicates. Significant differences represented by; $***P \leq 0.001$; $*P \leq 0.05$. Control (four replicates) = 4.14 log cfu/ml. Inoculate 30 min, ambient dry 60 min.

was 1.05 log cfu/ml *L. innocua*. The destruction was > 3.60 log cfu/ml *L. innocua* at all conditions except at 138 °C and one cycle. There was no visible thermal damage to the fruit. Final optimization on a commercial process for grapefruits, as for all the other products reported here, must include economic considerations of the process so either of the conditions chosen is a tentative optimum.

3.3. Beets

Growing in the ground, beets were found to be consistently high in naturally occurring bacteria. Four control samples had a mean APC count of 6.08 log cfu/ml (S.D. = 0.078). Therefore, beets were not inoculated. Total APC was used for investigation. The first experimental design was a 2^3 factorial, with steam temperature set to 127 and 138 °C, steam time set to 0.1 and 0.2 s, and one and two cycles used as the variable levels (data not shown). All three main factors were statistically significant, $P \leq 0.05$, with steam temperature highly statistically significant, $P \leq 0.001$. The total aerobic plate count for the high level of steam temperature (138 °C) was 3.85, for the high level of steam time (0.2 s) was 3.95, and for the high level of number of cycles was 3.93 log cfu/ml APC. With the control at 6.08 log, the bacteria destruction was 2.23, 2.15 and 2.15 log cfu/ml APC, respectively.

The next experimental design for beets (data not shown) increased the high steam temperature to 143 °C and set the low temperature to 138 °C. The other two variables were kept at 0.1 or 0.2 s for steam time and one and two cycles. Treatment samples consisted of four replicates. Steam time of 0.2 s was statistically significantly, $P \leq 0.05$, better than 0.1 s. Number of cycles was probably statistically significant, $P \leq 0.10$, two cycles better than one cycle. The mean bacteria count at the high level of steam time, 0.2 s, was 3.23 log cfu/ml APC. The mean bacteria count at the high level of number of cycles, two, was 3.29 log cfu/ml APC. With the control at 5.83 log cfu/ml APC (six replicates), the destruction was 2.60 log cfu/ml APC for 143 °C and 2.54 log cfu/ml APC for two cycles.

Table 5
2^3 Experimental design and ANOVA for beets

	−	+
A	138	143
B	0.2	0.3
C	1	3

Source	Mean Log cfu/ml	APC	
		Mean square	*F* value
A	3.38	3.044	9.80**
B	4.14	0.041	0.13
AB	3.67	3.781	0.001
C	3.40	4.689	15.09***
AC	3.03	0.025	0.08
BC	3.36	0.210	0.67
ABC	2.60	0.322	1.04
Error		0.311	

A = steam temperature, °C; B = steam time, s; C = number of cycles; Initial and intermediate vacuum times = 0.1 s; Final vacuum time = 0.3 s. Treatment samples consisted of four replicates. Significant differences represented by; $**P \leq 0.01$; $***P \leq 0.001$. Control (six replicates) = 6.00 log cfu/ml (S.D. = 0.216). APC = total aerobic plate count.

Steam temperature was kept at the same level in the next experimental design, Table 5. Steam time and number of cycles were increased. The variable levels and results are listed as described for Table 1.

Steam time was not statistically significant, so 0.2 s was chosen as the optimum steam time. Steam temperature was highly statistically significant, $P \leq 0.01$; 143 °C was better than 138 °C. Three cycles was highly statistically significant better than one cycle.

The mean bacteria count for the high level of steam temperature (A +, 143 °C) was 3.17 log cfu/ml APC. The mean bacteria count for the high level of number of cycles (C +, three cycles) was 3.09 log cfu/ml APC. The control using six replicates was 6.00 log cfu/ml APC. Therefore, the reduction in bacteria was 2.83 log cfu/ml APC at 143 °C (A +) and 2.91 log cfu/ml APC for three cycles (C +).

The optimum process parameters for beets were chosen as 143 °C, the maximum practical steam temperature in this prototype VSV intervention processor, steam time of 0.2 s, final vacuum time of 0.1 s and three cycles. Initial vacuum time was 0.1 s. Cycling more than three times might destroy more bacteria but we have observed the prototype VSV processor tended to cause abrasion and cutting damage to products when cycled more than three times. The damage is apparently caused by product getting into the advancing edge of the vacuum or steam ports in the platter valves.

3.4. Other fruits and vegetables

A similar study was made on papayas. Processing conditions should be very similar for other fruits and vegetables. Certainly there will be exceptions. Therefore, an even more narrow range was chosen for the initial process parameters for papayas. Papayas were inoculated with *L. innocua* for 10 min and dried in ambient air for 1 h. The optimum conditions found are listed in Table 6. Optimum steam conditions chosen were 138–143 °C for 0.2 s for two cycles. The destruction was 3.56 log cfu/ml *L. innocua*.

Other tropical fruits were tried at the general optimum conditions of 138 °C steam for 0.1 s, one, two and three cycles. Initial and intermediate vacuum times were 0.1 s and the final vacuum time was 0.3 s. The

Table 6
Application of the VSV surface intervention process to other fruits and vegetables

Commodity	Bacteria	Steam temp. (°C)	Steam time per cycle (s)	No. of cycles	Control Log (cfu/ml)	Treated Log (cfu/ml)	Destruction Log (cfu/ml)
Papayas	*L. innocua*	138/143	0.2	1/2	5.2	1.6	3.6
Mangoes	*L. innocua*	138	0.1	2	5.4	1.4	4.0
Avocados	*L. innocua*	138	0.1	2	4.1	1.0	3.1
Kiwis	*L. innocua*	138	0.1	3	6.4	1.6	4.7
Bananas	*	104	0.1	1			Mutilated
Carrots	APC	138	0.1	3	5.7	1.6	4.2
Cucumbers	APC	138	0.1	3	5.4	1.6	3.8
Peaches	*L. innocua*	138	0.1	2	5.0	1.4	3.6
Cauliflower	*	127	0.1	1			Color change
Broccoli	*	116	0.1	1			Color change
Peppers	*	116	0.1	1			Mutilated

Initial and intermediate vacuum times = 0.1 s. Final vacuum time = 0.3 s.
*No microbiology analyses were performed on products that were damaged.

results for kiwis, mangoes, and avocados are listed in Table 6. All three were inoculated with *L. innocua* for 10 min and ambient dried for 1 h. The log destruction for kiwis was 4.7, for mangoes 4.0 and for avocados 3.1.

Another tropical crop, bananas, was tried. However, the process destroyed them. The peel split and the fruits darkened immediately. Milder conditions were tried with green bananas, Table 6. At 104 °C, for 0.1 s. and one cycle, the bananas were still destroyed. Bananas could not be successfully processed. No microbiological analyses were performed on products that were damaged.

Other products that could not be successfully processed were peppers and broccoli and cauliflower, Table 6. When subjected to vacuum, the peppers exploded. The delicate florets on broccoli turned a bright green, indicative of blanching or heat treatment. Although the flower part of cauliflower was essentially unscathed, the stalk and the remnants of the leaves turned bright green as in blanching.

Other fruits and vegetables were tried at the general optimum conditions as stated above. The results are listed in Table 6. Carrots were run without inoculation. The bacteria destruction was 4.2 log cfu/ml APC. Although cucumbers had a relatively high background bacterial count (APC = 4.55 log cfu/ml, S.D. = 0.0929) they were inoculated for 10 min with *L. innocua* to increase the bacteria count and ambient dried for 1 h. There was a 3.8-log destruction with three cycles. Peaches were inoculated for 10 min with *L. innocua* and ambient dried for 1 h. Using two cycles, the bacteria destruction was 3.6 log cfu/ml for *L. innocua*.

4. Conclusions

The VSV surface intervention process destroys bacteria, *L. innocua* and APC, on and in the surface of fruits and vegetables. Optimum processing conditions chosen, subject to constraints, varied somewhat for specific fruits and vegetables but were generally steam temperature of 138–143 °C, for 0.1–0.2 s, initial and intermediate vacuum times of 0.1 s, and 0.3 s final vacuum times. Cycling generally improved the bacteria destruction. Successful application of the process is product specific. Most of the produce tested achieved bacteria destruction, *L. innocua* and APC, of 3–5 log cfu/ml with no visual thermal damage to the product surface whereas, some produce, namely, bananas, cauliflower, peppers, and broccoli, were damaged and unsuitable for this process.

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